

## Report

# Interkinetic Nuclear Migration Is a Broadly Conserved Feature of Cell Division in Pseudostratified Epithelia

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## Summary

Animal development requires tight integration between the processes of proliferative growth and epithelial morphogenesis, both of which play out at the level of individual cells. In this respect, not only must polarized epithelial cells assume complex morphologies, these distinct forms must be radically and repeatedly transformed to permit mitosis. A dramatic illustration of this integration between epithelial morphogenesis and cell proliferation is interkinetic nuclear migration (IKNM), wherein the nuclei of pseudostratified epithelial cells translocate to the apical epithelial surface to execute cell division [1, 2]. IKNM is widely considered a hallmark of pseudostratified vertebrate neuroepithelia, and prior investigations have proposed both actomyosin- and microtubule-dependent mechanisms for apical localization of the mitotic nucleus [3–8]. Here, using comparative functional analysis in arthropod and cnidarian systems (*Drosophila melanogaster* and *Nematostella vectensis*), we show that actomyosin-dependent IKNM is likely to be a general feature of mitosis in pseudostratified epithelia throughout Eumetazoa. Furthermore, our studies suggest a mechanistic link between IKNM and the fundamental process of mitotic cell rounding.

## Results and Discussion

A conserved feature of metazoan cell division is a rounding-up phase prior to spindle assembly, which occurs in systems as diverse as cultured potoroo kidney cells and the pseudostratified epithelia of *Drosophila* imaginal discs [9–11]. Cytoskeletal mechanisms of cell rounding have been probed with *in vitro* experiments, which have defined essential functions for RhoA and actin dynamics at the cell cortex [9, 12, 13]. Recent studies have also explored the contribution of membrane recycling to surface area changes during cell rounding in vertebrate tissue culture [14], as well as critical roles for the ERM-domain protein moesin in controlling rounding and cortical stiffness in *Drosophila* tissue culture cells [15, 16]. Still, despite these advances, precisely how mitotic cell rounding is coordinated with the maintenance of epithelial architecture remains poorly understood.

Perhaps the most conspicuous example of the morphological transformations associated with epithelial cell mitosis is interkinetic nuclear migration (IKNM), often considered a hallmark of pseudostratified vertebrate neuroepithelia [5–7, 17, 18]. First described in 1935 by F.C. Sauer in the pig neural tube, IKNM is the process by which mitotic nuclei move to the apical epithelial surface to execute cell division [1]. Sauer

envisioned IKNM as monotonic, cyclical oscillations of the nucleus between apical positions in mitosis and basal positions in interphase, and studies since have pointed to a key role for microtubule-based force generation in this process [4, 7, 8]. Recently, through time-lapse analyses and pharmacological inhibitor experiments, actomyosin contractility was shown to be the primary motile force in IKNM in retinal epithelia of the zebrafish *Danio rerio* [5]. These studies also favor the interpretation of IKNM as a stochastic process, challenging Sauer's original conception of monotonic nuclear movements. In the present analysis, we address two main questions. First, if IKNM is a hallmark of vertebrate neuroepithelia, how is the division of pseudostratified epithelial cells controlled in nonneural and nonvertebrate systems? Second, is there a mechanistic link between mitotic rounding and IKNM, which also relies on actin dynamics at the cell cortex?

## A Septate Junction-Delimited Mitotic Zone in *Drosophila* Wing Discs

*Drosophila* imaginal discs (Figure 1A; [19]) are flattened epithelial sacs comprised of apposed squamous and pseudostratified cell layers. In the pseudostratified layer, mitotic figures identified by anti-phosphohistone H3 (anti-PH3) labeling were precisely localized to the plane of Discs large (DLG) accumulation at the septate junctions (Figure 1B). Mitotic progression occurred through a stereotyped cell rounding process at the apical epithelial surface, beginning with expansion of the junctional lattice and subsequent movement of mitotic chromatin into alignment with the septate junctions (Figure 1C; [10]). In xz sections, rounded cells were restricted to apical positions (Figure 1D, yellow arrows). In contrast, interphase cells marked with the *flp>FRT* technique [20] spanned the width of the epithelium (Figure 1E), with nuclei heterogeneously distributed in a medial domain (Figures 1E and 1F). Confirming that the rounded cells were mitotic figures, they exhibited anti-PH3<sup>+</sup> nuclei predominantly observed within 10  $\mu$ m of the apical epithelial surface, in a mitotic zone (MZ) delimited by the septate junctions (Figure 1G). Consistent with the maintenance of epithelial junctions throughout division of Madin-Darby canine kidney cells [21, 22], these results imply that mitotic cells round up apically as a result of the continuous integrity of adhesive junctions. In addition, our observations define an IKNM-like process in *Drosophila* imaginal discs, where apical cell rounding correlates with translocation of prophase nuclei from the medial zone into the MZ. Extending the similarity to the stochastic form of IKNM described in zebrafish [5, 23], S phase nuclei labeled with a short pulse of EdU showed no obvious apical or basal bias within the medial nuclear zone (see Figure S1A available online).

## Actomyosin Dynamics Correlate with Prophase Entry and Apical Cell Rounding

While analyzing the localization of anti-PH3<sup>+</sup> nuclei, we observed that mitotic cells possessed an intensely F-actin-rich basal process that formed in early prophase and was present throughout mitosis (Figure 1H, yellow arrows). We also observed long, thin basal extensions of apically rounded mitotic cells in single-cell clones labeled with GFP (Movie S1).

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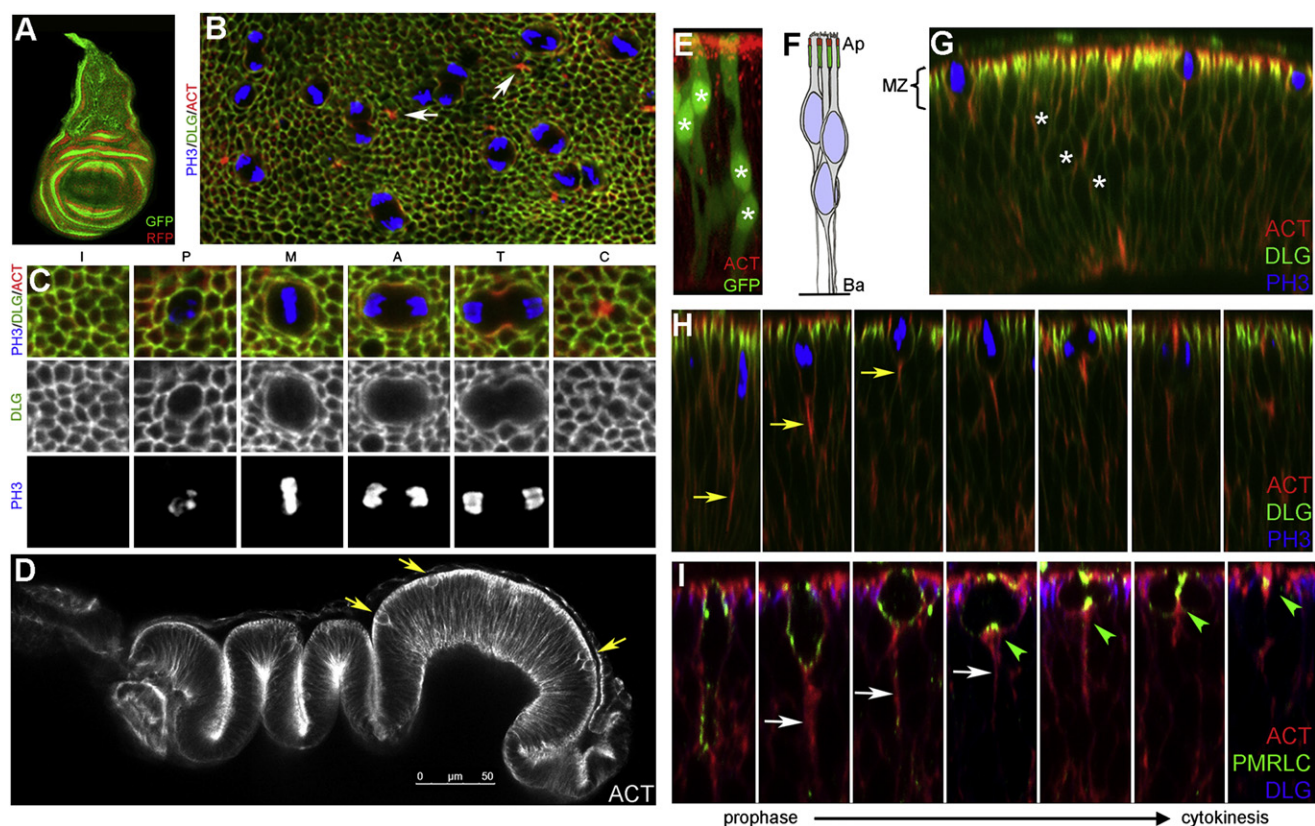


Figure 1. Interkinetic Nuclear Migration-like Mitotic Cell Behavior in the *Drosophila* Wing Disc

(A) *neuroglian-GFP;histone H2-RFP* third-instar wing imaginal disc.

(B) Anti-phosphohistone H3 (PH3)<sup>+</sup> mitotic figures (blue) colocalize with the plane of the septate junctions as labeled by Discs large (DLG, green). Phalloidin staining of F-actin (ACT) is red. Terminal stages of cytokinetic furrow contraction are visible as F-actin-rich foci, also confined to the plane of the septate junctions (white arrows).

(C) Stages of mitosis (interphase, prophase, metaphase, anaphase, telophase, and cytokinesis) from fixed samples labeled for DLG (green), mitotic chromatin (blue), and F-actin (red). Note the continuous localization of mitotic figures to the plane of the septate junctions.

(D) Phalloidin-stained cross-section through a wing imaginal disc. Yellow arrows indicate rounded and presumably mitotic cells at the apical epithelial surface.

(E) Stochastically labeled GFP<sup>+</sup> clones (green) illustrate the variability in nuclear positioning (white asterisks) in interphase cells.

(F) Schematic representation of interphase nuclei relative to the polarized cell-cell junctions (adherens junctions, red; septate junctions, green; nuclei, blue; Ap, apical; Ba, basal).

(G) Whereas interphase nuclei occupy medial positions within the ~50  $\mu$ m-thick epithelium (white asterisks), rounded cells and anti-PH3<sup>+</sup> mitotic figures (blue) are restricted to the DLG-delimited mitotic zone (MZ, green).

(H) In fixed discs stained as above, intense basal F-actin accumulation is first observed in prophase cells (leftmost panel, yellow arrow) and persists throughout mitosis.

(I) In discs stained for anti-DLG (blue) and F-actin (red), anti-p-MRLC (green) accumulates at the cortex of early prophase figures and persists throughout mitotic rounding. Notably, p-MRLC is not detected in the F-actin-rich basal process (white arrows). Uniform cortical p-MRLC is lost or redistributed during formation and progression of the contractile ring (green arrowheads).

These findings indicate that, during mitotic rounding, imaginal disc cells can maintain some connectivity with the basal side of the epithelium despite the dramatic apical translocation of both the nucleus and bulk cytoplasm.

We next measured myosin II activity using a phospho-myosin regulatory light chain 2 antibody (anti-p-MRLC). Most interphase cells in the wing imaginal disc exhibited little or no cortical p-MRLC enrichment (Figures S1C and S1D). By contrast, strong p-MRLC staining was observed at the cell cortex concomitant with the earliest signs of mitotic rounding (Figure 1I). Uniform cortical localization was maintained through cell rounding until metaphase and then became concentrated in the contractile ring during cytokinesis (green arrowheads in Figure 1I and Figures S1E and S1F). In mitotic cells, p-MRLC was excluded from the basal process (white arrows in Figure 1I and Figure S1F),

indicating that the cortex of the rounded cell body has distinct molecular properties from the basal extension. Although the contribution of actomyosin contractility to cytokinesis is well established, our observations suggest an additional, earlier function in mitotic rounding of polarized epithelial cells.

#### Cell Rounding and Apical Translocation of the Mitotic Nucleus Require Cortical Contractility and Rho Kinase Activity

Previous studies in vertebrate neuroepithelia suggest that IKNM could be driven by either actomyosin contractility [5, 24] or microtubule dynamics [7, 8, 25]. We therefore used an ex vivo pharmacological assay to determine the requirement for different cytoskeletal systems in the apical movement of prophase nuclei into the MZ of the *Drosophila* wing disc



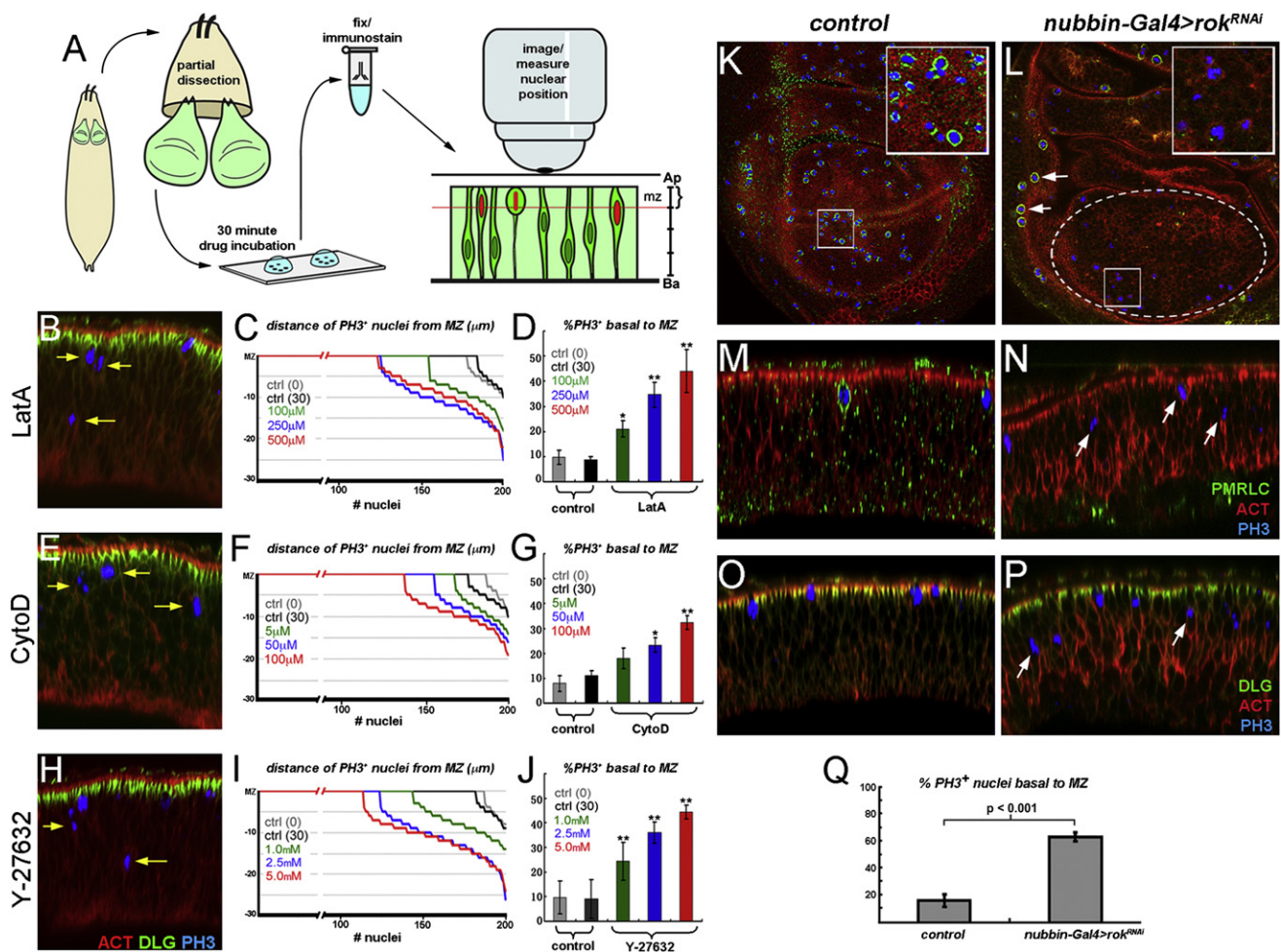


Figure 2. Rho Kinase and Cortical Contractility Are Required for Nuclear Translocation to the Mitotic Zone

(A) Experimental design for cytoskeletal inhibitor studies.  
 (B) Latrunculin A (LatA) treatment caused anti-PH3<sup>+</sup> nuclei to accumulate basal to the MZ (yellow arrows).  
 (C) Distance of 200 anti-PH3<sup>+</sup> nuclei from the MZ for control discs (uncultured and 30 min of culture) compared with discs treated for 30 min with 100, 250, and 500 μM LatA.  
 (D) Percentages of anti-PH3<sup>+</sup> nuclei outside the MZ following LatA treatment (n = 300 nuclei per condition).  
 (E and F) CytoD treatment (at 5, 50, and 100 μM) disrupted apical translocation of mitotic nuclei in a manner similar to LatA.  
 (G) Percentage of PH3<sup>+</sup> nuclei outside the MZ for CytoD treatments (n = 300 nuclei per condition).  
 (H and I) Effects of Y-27632 (at 1, 2.5, and 5 mM) on the positions of 200 anti-PH3<sup>+</sup> nuclei.  
 (J) Percentage of PH3<sup>+</sup> nuclei outside the MZ following treatment with Y-27632 (n = 300 nuclei per condition). Statistical analyses were performed with the Mantel-Haenszel test using controls cultured for 30 min.  
 (K–N) Third-instar wing imaginal discs stained for phalloidin (F-actin), anti-p-MRLC, and anti-PH3.  
 (K) *UAS-dicer-2;nubbin-Gal4* control disc showing rounded mitotic cells specifically labeled with anti-p-MRLC. Box in main image indicates position of inset.  
 (L) *nubbin-Gal4>rok<sup>RNAi</sup>* wing discs display a severe reduction of anti-p-MRLC staining as well as a reduction in the number of apically rounded cells within the *nubbin-Gal4* expression domain (dotted line). Note that mitotic cells outside the *nubbin-Gal4* domain exhibit normal anti-p-MRLC staining (white arrows).  
 (M) xz section of a control disc showing cortical anti-p-MRLC staining in PH3<sup>+</sup> cells.  
 (N) In *nubbin-Gal4>rok<sup>RNAi</sup>* discs, cortical anti-p-MRLC is eliminated in PH3<sup>+</sup> cells (white arrows).  
 (O) *UAS-dicer-2;nubbin-Gal4* control disc.  
 (P) *nubbin-Gal4>rok<sup>RNAi</sup>* disc showing numerous anti-PH3<sup>+</sup> nuclei (blue) basal to DLG accumulation at the septate junction (green).  
 (Q) Percentage of PH3<sup>+</sup> nuclei out of the MZ in *nubbin-Gal4>rok<sup>RNAi</sup>* discs (n = 450 nuclei per condition). Statistical analysis was performed using Student's t test.

\*p < 0.0001; \*\*p < 0.00001. Error bars indicate standard deviation.

(Figure 2A). Uncultured control discs exhibited approximately 90% of anti-PH3<sup>+</sup> nuclei within the MZ, specifically defined as from the cell apex to 4 μm basal to the DLG domain (n = 300 nuclei). Similarly, 91% of anti-PH3<sup>+</sup> nuclei were within the MZ in control discs cultured for 30 min (n = 300 nuclei). Generally, anti-PH3<sup>+</sup> nuclei basal to the septate junctions were in

prophase, whereas metaphase and anaphase figures were restricted to the plane of the MZ.

To test the requirements for actin polymerization, we introduced latrunculin A (LatA) at three concentrations during a 30 min culture. Disrupting actin polymerization significantly increased the percentage of anti-PH3<sup>+</sup> nuclei at abnormally

basal positions with the epithelium, suggesting that the cells entered prophase but that the apical translocation was either blocked or delayed (Figures 2B–2D). LatA treatment also increased the average distance of these basal anti-PH3<sup>+</sup> nuclei from the MZ (Figure 2C; Table S1). The majority of these basal nuclei exhibited multiple foci of intense anti-PH3 staining, contrasting with the more diffuse nuclear signal in controls. Furthermore, only prophase figures were detected basal to the MZ. Similar results were obtained in discs treated with cytochalasin D (CytoD; Figures 2E–2G; Table S1). Importantly, drug effects in these assays were partially masked by the large percentage of anti-PH3<sup>+</sup> nuclei already in the MZ at the time of drug application; among these, we observed a significant mitotic arrest (Table S2). Nevertheless, disruption of actin dynamics with either LatA or CytoD caused a significant increase in the number of anti-PH3<sup>+</sup> nuclei basal to the MZ (Figures 2D and 2G).

Actomyosin contractility is a key force-generating mechanism in eukaryotic cells. In epithelia, Rho GTPases regulate myosin II activity via Rho kinase-dependent phosphorylation of the MRLC and inactivation of myosin phosphatase [26, 27]. RhoA has been implicated in the mitotic rounding of HeLa cells via Rho kinase [12], which has also been shown to phosphorylate myosin during thrombin-induced rounding of human tissue culture cells [28]. We therefore tested the effect of the Rho kinase inhibitor Y-27632 [29, 30] on cell rounding and nuclear movement during ex vivo culture. Treatment of imaginal discs with Y-27632 blocked p-MRLC accumulation in mitotic cells (Figures S2A–S2D and S2G–S2J) and caused a more severe disruption of apically directed prophase movements than either CytoD or LatA (Figure 2H–2J). We again analyzed the position of anti-PH3<sup>+</sup> nuclei and found 44% basal to the MZ, compared with 9% for controls (at 5 mM Y-27632; Figure 2J). Similar to both LatA- and CytoD-treated discs, these basal anti-PH3<sup>+</sup> nuclei tended to be further from the MZ than those observed in controls (Figure 2I; Table S1). Suggesting that the basal anti-PH3<sup>+</sup> nuclei were in prophase, they were not associated with  $\gamma$ -tubulin-positive centrosomes (Figures S3A and S3B).

Together, the experiments above indicate that actin dynamics and Rho kinase play a critical function in the apical translocation of mitotic nuclei during wing disc IKNM. To confirm this genetically, we expressed a *Rho kinase* RNAi construct (*rok<sup>RNAi</sup>*) under the control of a Gal4 driver specific to the wing blade territory (*UAS-dicer-2;nubbin-Gal4*). Wing discs expressing *rok<sup>RNAi</sup>* exhibited mild morphological defects, and the anti-p-MRLC staining normally associated with mitotic cells was severely and specifically reduced within the *nubbin-Gal4* expression domain (Figures 2K and 2L). Consistent with the Y-27632 experiments, anti-PH3<sup>+</sup> nuclei accumulated at medial positions in the *rok<sup>RNAi</sup>* expression domain (Figures 2M and 2N). In these discs, more than 60% of anti-PH3<sup>+</sup> nuclei were basal to the MZ, compared to 15% for controls (Figures 2O–2Q). In addition, apical cell rounding was strongly affected (Figures S3C–S3F). Together, these results are most consistent with the view that Rho kinase activates cortical contractility at the onset of prophase, resulting in cell rounding and the apical translocation of the mitotic nucleus. One important consideration is that Rho kinase could utilize multiple effectors in addition to myosin. Moesin, for example, which is regulated by RhoA/Rho kinase [31–33] and is implicated in mitotic cell rounding [15, 16], is also likely to play an important role.

#### Microtubule Dynamics during Wing Disc IKNM

Microtubules play a central role in nuclear positioning in a wide variety of eukaryotic cells [34]. In the *Drosophila* eye disc,

microtubule motors control nuclear positioning in postmitotic neurons [35], and previous work on vertebrate neuroepithelia indicates a function for microtubules in regulating IKNM [7, 8, 25]. Indeed, in the most thickened regions of the *Drosophila* wing disc, interphase cells exhibit an intense apical accumulation of microtubules oriented parallel to the apico-basal axis [36, 37]. In cross-sections at the apical epithelial surface, polymerized microtubules formed a meshwork filling the polygonal profile of each cell (Figure 3A, asterisks), similar to what has been described in pupal stages [38]. Intriguingly, this apical mesh was cell-autonomously disassembled at prophase entry (Figure 3A, arrow). As the prophase nucleus moved into the MZ, polymerized microtubules reappeared in association with the forming mitotic spindle (Figure 3A, arrowhead). During cytokinesis, midbody microtubules persisted in the narrowed bridge between the coequal daughters and progressively turned basally to become oriented along the apicobasal axis of the cell, perhaps reconstituting the interphase arrays (Figures S4A–S4F). These events were more clearly observed in xz sections through fixed wing discs, with loss of the apical microtubules in prophase (Figures 3B and 3C) followed by formation of the mitotic spindle (Figures 3D and 3E) and the appearance of apicobasally aligned midbody microtubules (Figures 3F–3H).

To test the function of microtubule dynamics in mitotic cell rounding and IKNM, we used the same ex vivo culture approach for the application of three different concentrations of paclitaxel (Figures 3I–3K) and colchicine (Figures 3L–3N). Surprisingly, inhibiting microtubule dynamics had comparatively minor effects in our assay, even though both drugs caused significant prophase arrest (Table S3) and colchicine visibly disrupted microtubule organization (Figures S2E, S2F, S2K, and S2L). Interestingly, stabilizing microtubules with paclitaxel had a stronger effect than colchicine treatment, suggesting that disassembly of the interphase microtubules may be important for efficient apical translocation of the prophase nucleus. Still, given the limitations of pharmacological inhibitor treatments, further studies are needed to define the precise function of microtubule dynamics in *Drosophila* IKNM.

#### The Ancient Origin of IKNM: Beyond Bilateria

Our descriptive and functional studies in *Drosophila* indicate a prominent role for Rho kinase and cortical contractility in driving cell rounding and apical translocation of the mitotic nucleus. In this respect, IKNM in *Drosophila* imaginal discs is similar or identical to what is observed in some vertebrate neuroepithelia (e.g., [5]). To test whether IKNM is a widespread mechanism for pseudostratified epithelial cell division, we also analyzed cell proliferation during development of a cnidarian, the sea anemone *Nematostella vectensis*. Cnidaria and Bilateria diverged about 500 million years ago, prior to the emergence of centralized nervous systems (e.g., [39]). *Nematostella* larvae exhibit a pseudostratified ectodermal layer during the planula stage of development (Figures 4A and 4B). Using confocal microscopy, we observed rounded mitotic cells with cortical enrichment of F-actin at the apical epithelial surface (Figure 4C). In transverse sections, these mitotic figures closely resembled those observed in *Drosophila* imaginal discs, including the presence of an F-actin-rich basal process (Figures 4D–4F'). Also similar to *Drosophila*, interphase nuclei occupied a densely packed medial zone (Figures 4E and 4F) and anti-PH3<sup>+</sup> nuclei localized to an apical MZ (Figure 4G). We did not detect an obvious bias in the position of S phase nuclei at this developmental stage

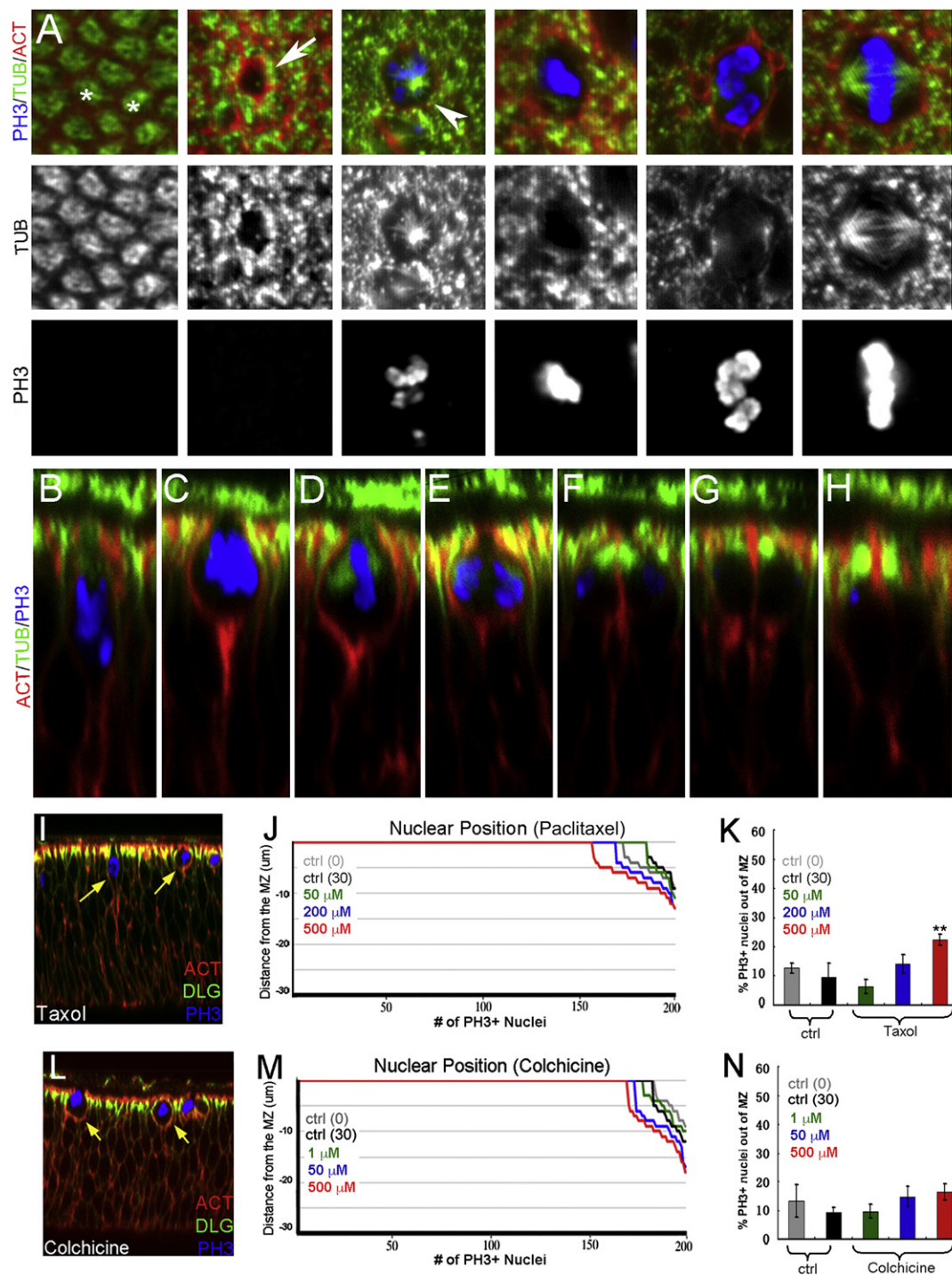


Figure 3. Microtubule Dynamics during Mitosis in the *Drosophila* Wing Disc

(A) Apices of fixed wing disc cells exhibit a diffuse microtubule web during interphase (white asterisks). As cells enter prophase and begin to round up, the apical microtubule web disappears (white arrow). The mitotic spindle begins to form in subsequent stages (white arrowhead).

(B–H) In xz sections through fixed discs, apical microtubules orient along the apicobasal axis. Apical anti-tubulin staining (green) diminishes in early prophase (B) and vanishes altogether as the anti-PH3<sup>+</sup> nucleus (blue) reaches the MZ (C). After formation of the mitotic spindle (D and E) and cytokinesis, the midbody microtubules turn basally, ultimately becoming oriented along the apicobasal axis of the daughter cells (F–H).

(I–K) Paclitaxel treatments were sufficient to cause a mitotic arrest (Table S3) but did not strongly affect the apicobasal positions of mitotic nuclei (yellow arrows in I) or mitotic cell rounding.

(J) Plotted positions of 200 nuclei relative to the MZ for 50, 200, and 500 μM paclitaxel.

(K) Percentages of anti-PH3<sup>+</sup> nuclei basal to the MZ following paclitaxel treatments (n = 300 nuclei per condition). Statistical analyses in (K) and (N) were performed with the Mantel-Haenszel test using controls cultured for 30 min. \*\*p < 0.0001. Error bars indicate standard deviation.

(L–N) Colchicine treatment (at 1, 50, and 500 μM) caused a mitotic arrest (Table S3) but did not strongly affect the apicobasal positions of mitotic nuclei or mitotic cell rounding (yellow arrows in L).



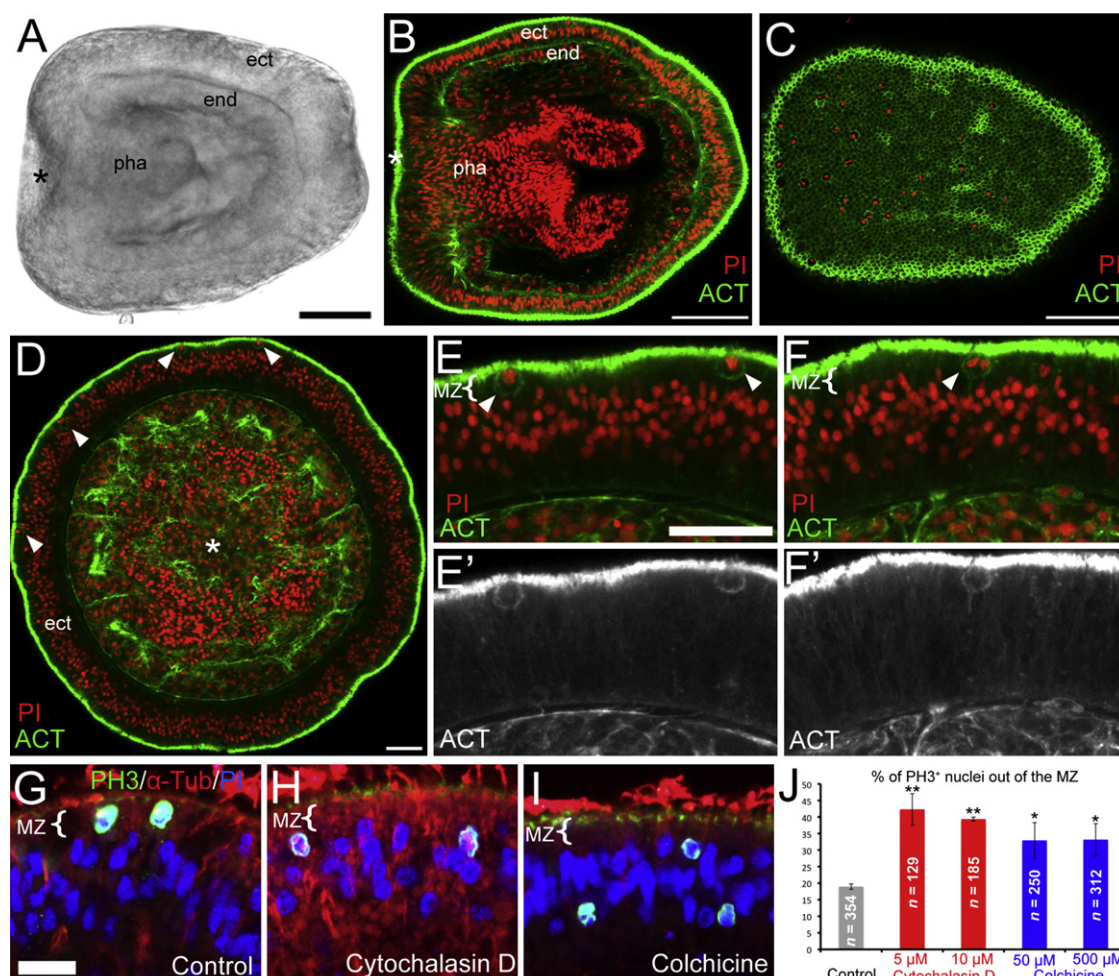


Figure 4. Interkinetic Nuclear Migration in the Ectoderm of *Nematostella vectensis*

(A) Differential interference contrast image of a 4-day-old planula larvae showing ectoderm (ect), endoderm (end), blastopore/oral pole (asterisk), and pharynx (pha). Scale bars represent 50  $\mu$ m in (A)–(C).  
(B) *Nematostella* larvae stained with phalloidin to label F-actin (ACT, green) and propidium iodide to label nuclei (PI, red).  
(C) As in *Drosophila* (e.g., Figure 1B), the apical surface of the planular ectoderm exhibits rounded cells with apically localized nuclei.  
(D) Transverse view showing apical rounding of cells (white arrowheads). Scale bars represent 25  $\mu$ m in (D) and (E).  
(E–F) Detailed view of the ectoderm. Rounded cells in the MZ exhibit enriched cortical F-actin (white arrowheads).  
(G) Apical localization of anti-PH3<sup>+</sup> nuclei (green) in the ectoderm of control larvae stained with anti- $\alpha$ -tub (red) and PI (blue). Scale bar represents 10  $\mu$ m.  
(H and I) CytoD-treated (H) and colchicine-treated (I) animals exhibit anti-PH3<sup>+</sup> nuclei in abnormal positions basal to the MZ.  
(J) Effects of CytoD and colchicine on the position of anti-PH3<sup>+</sup> nuclei. Statistical analyses were performed with the Mantel-Haenszel test using controls cultured in dimethyl sulfoxide. \* $p < 0.0001$ ; \*\* $p < 0.00001$ . Error bars indicate standard deviation.

(Figure S1B), although a basal positional bias may exist at later stages of development (data not shown).

To assess the contribution of different cytoskeletal elements to *Nematostella* IKNM, we treated 4-day-old planula larvae with CytoD, Y-27632, or colchicine and then analyzed the position of anti-PH3<sup>+</sup> nuclei with respect to the MZ. In controls, approximately 20% of anti-PH3<sup>+</sup> nuclei were observed basal to the MZ ( $n = 354$ ). Consistent with our results in *Drosophila*, disrupting actin polymerization with CytoD nearly doubled the percentage of anti-PH3<sup>+</sup> nuclei in the medial zone (Figures 4H and 4J). An even stronger effect was observed when 2.5 mM Y-27632 was used to inhibit Rho kinase activity (Figures S3G–S3J). These animals also exhibited intrusion of interphase nuclei into the MZ (Figure S3H), an effect we did not observe in *Drosophila*. Also contrasting with our results in *Drosophila*, disrupting microtubules with colchicine increased the number of anti-PH3<sup>+</sup> nuclei at abnormally basal positions (Figures 4I

and 4J). This indicates a role for microtubules in apical translocation of the mitotic nucleus and suggests that both cortical contractility and microtubule dynamics contribute to IKNM-like movements in *Nematostella*.

## Conclusions

The results presented here have two main implications. First, we describe IKNM-like processes in the pseudostratified epithelia of arthropods and cnidarians, indicating that this mode of cell division is widespread and not a unique feature of vertebrate neuroepithelia. Mechanistically, our findings are most consistent with the view that IKNM is a highly conserved process primarily driven by cortical contractility associated with mitotic cell rounding at prophase entry but that it has been specialized in some instances to require microtubule-dependent nuclear movements ([7, 8, 40]; Figures 4I and 4J). Second, the deep evolutionary conservation of IKNM-like

mitotic behavior throughout Eumetazoa suggests the existence of strong constraints on the mechanism of cell division in pseudostratified epithelia. We favor a model wherein IKNM is required to restrict cell divisions to the plane of the apical junctions, thereby ensuring continuous monolayer integrity of proliferating epithelial sheets.

#### Supplemental Information

Supplemental Information includes four figures, three tables, Supplemental Experimental Procedures, and one movie and can be found with this article online at [doi:10.1016/j.cub.2011.02.002](https://doi.org/10.1016/j.cub.2011.02.002).

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